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An Efficient Heating-Detection Chamber for Vapor Phase Fluorescence TLC

Robert J. Maxwell*

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1 Introduction

The concept of vapor phase fluorescence (VPF) was first introduced by *Segura* and *Gotto* in 1974 [1]. They reported that fluorescence could be induced in many organic compounds having no native fluorescence by heating the substance spotted on a TLC plate in the presence of ammonium bicarbonate. The technique has since been applied to a number of organic chemical classes including lipids, steroids, carbohydrates, and antibiotics. The original VPF work was done with a 15 L sealed tank as the heating chamber [1]. Since then other workers have used a variety of silicone grease sealed containers to expose TLC plates to vapors produced from the thermal decomposition of ammonium bicarbonate [2], including a commercial unit designed for this purpose. We have tested many of these designs, including the commercial apparatus, in the course of our studies of the VPF method for the detection of polycyclic ether antibiotics, and have had difficulties with all of them. For instance, TLC development tanks develop thermal stresses at the temperatures required for these experiments and only a limited number of these tanks are available with leak-proof seals. The commercial device does not have a sealed chamber, allowing the vapor to dissipate during the heating period. The lack of a suitable chamber for VPF TLC could potentially limit development of this important technique for TLC detection. We have therefore designed a leak-proof VPF chamber that will hold up to six 10 × 10 cm TLC plates that is easily assembled from parts readily available from laboratory supply houses.

2 Experimental

2.1 Chemicals, TLC Plates, and Spectrophotometry

Monensin sodium was obtained from Lilly Research Laboratories (Greenfield, IN). Lipid standards (**Figure 3**) were from Sigma Chemical Co. (St. Louis, MO), Silica gel 60 HPTLC plates were from E. Merck (Cherry Hill, NJ). Fluorescence spots were measured using a Camag TLC Scanner II (Wrightsville Beach, NC) with a 366 nm excitation wavelength from a mercury lamp source and fluorescence at 400 nm (cut-off filter). The slit width was 0.2 mm and the slit height was 3.0 mm. The VPF visualizing chamber was obtained from Analtech, Inc. (Newark, DE).

R. J. Maxwell, U.S. Department of Agriculture, ARS, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia PA 19118, USA.

2.2 VPF Heating Apparatus

1. Resin kettle, 2000 ml, part no. 11-847-10 C Fisher Scientific Co. (King of Prussia, PA). 2. Perforated shelf, constructed locally from a 12.7 cm diameter stainless steel sheet with approximately 13 mm diameter hole pattern. 3. Zinc plated steel test tube rack 233 × 121 × 68 mm, part no 9226-U 25, A. H. Thomas Co. (Philadelphia, PA) cut to fit opening (10 cm) of the resin kettle and attached to perforated shelf with aluminum wire. 4. Teflon gasket, 170 mm o.d. × 130 mm i.d. cut from 12″ × 12″ × 1/32″ sheeting, Bolab (Lake Havasu City, AZ). 5. Pyrex glass cover, 165 mm diameter, cut from 200 × 200 × 4 mm glass plate, Alltech Associates (Deerfield, IL). 6. Cover clamp, part no. 11-847-30 B, Fisher Scientific Co. (King of Prussia, PA) or part no. 4968-K 20 A. H. Thomas Co. (Philadelphia, PA). 9. Heating mantle, Glas-Col, part no. 11-847-15 C, Fisher Scientific Co. (King of Prussia, PA).

3 Results and Discusion

Our initial VPF HPTLC heating experiments were carried out with the commercial VPF chamber. We noted considerable differences in the day to day use of this apparatus. Moreover, during extended heating experiments it was necessary to repeatedly recharge the unit with NH_4HCO_3 to maintain an atmosphere of ammonia over the TLC plates because the apparatus is not vapor tight. Because of difficulties in reproducibility encountered with this device, we concluded that acceptable results with the VPF technique only could be obtained with a sealed chamber. We next attempted to adapt commonly available TLC and other types of chromatography tanks to our purposes, and again encountered similar difficulties. Originally *Segura* and *Gotto* [1] used a 15 L tank for their work, a vessel too large to be practical. *Kupke* and *Zeugner* [2] employed a sandwich-type apparatus sealed with silicone grease. We examined several types of chromatography jars and TLC development tanks having ground glass tops, which we fitted with glass covers and sealed with either silicone grease or silicone gaskets. This approach was not successful because of several distinct problems. Most of these cylinders or TLC tanks do not have precision ground surfaces and allow vapor to escape regardless of the method used to seal the containers. Additionally, the ground glass tops of the container walls are typically narrow (3–5 mm) further precluding the possibility of making effective seals. We were able to seal some of the TLC tanks that we tested with silicone grease; however these tanks could only be heated once or twice because they are not tempered and soon developed cracks.

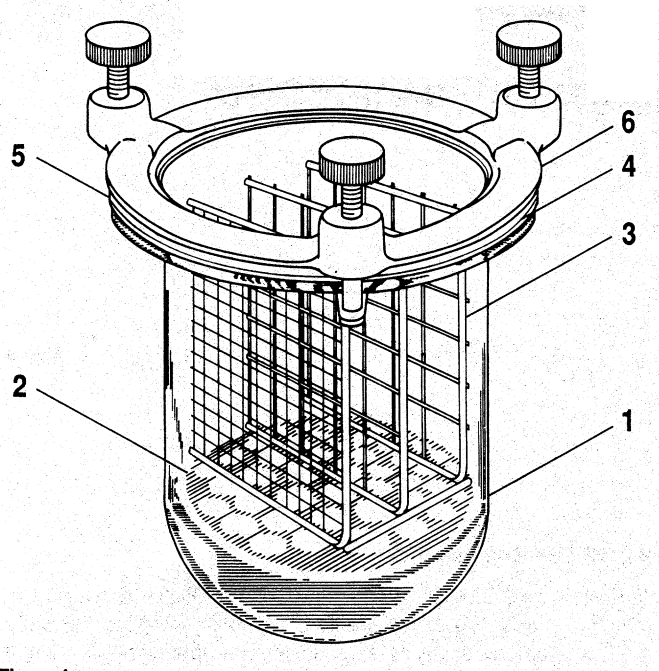

Figure 1

1 Resin kettle; 2 Perforated shelf; 3 Test tube rack; 4 Teflon gasket; 5 Pyrex glass cover plate; 6 Cover clamp.

Because of the problems encountered with conventional tanks and jars, we designed a VPF TLC heating chamber around a 2000 ml resin kettle. These readily available kettles are made of Pyrex glass and have wide (14 mm) flanges (**Figure 1**). When the kettle is fitted with a Pyrex glass cover, teflon gasket, and spring loaded clamp the unit makes a grease-free, vapor-tight seal. The 10 × 10 cm TLC plates are held (maximum six) on a basket made from a stainless steel plate and a zinc-coated test tube rack (Figure 1). This basket may be conveniently raised or lowered to facilitate the placement or removal of TLC plates. If heating this device in an oven is not convenient, the unit may be heated on the lab bench in a Glas-Col heating mantel designed to hold the resin kettle (Experimental). In this case, the temperature inside the kettle can be monitored with a stainless steel thermometer with a glass cover. Additionally, when the heating chamber is not in use, previously developed TLC plates may be stored in it with small amounts of NH_4HCO_3 to preserve the fluorescent spots.

This heating device has been used with several classes of compounds and has repeatedly given uniform, reproducible results. In comparison studies, serially diluted monensin sodium standards were statically spotted on two Merck silica HPTLC plates and heated separately in the resin kettle and in the commercial VPF Chamber (**Figure 2**). Note the low sensitivity and lack of linearity (R^2 = 0.994 heating chamber *vs.* R^2 = 0.912 commercial VPF chamber) depicted in the chromatogram of the monensin heated in the commercial unit (Figure 2b). Similarly, a series of lipid standards were statically spotted and heated using the same conditions as described above **(Figure 3).** From the Figure it is clear that all six lipid classes had enhanced responses when heated in the resin kettle chamber (Figure 3a) over those observed for the same compounds heated in the commercial unit (Figure 3b). The most dramatic response difference was found for L-α-phosphatidylcholine, where the response was 198% greater for the sample reacted in the resin kettle heating chamber (Figure 3a) than that heated in the commercial unit (Figure 3b).

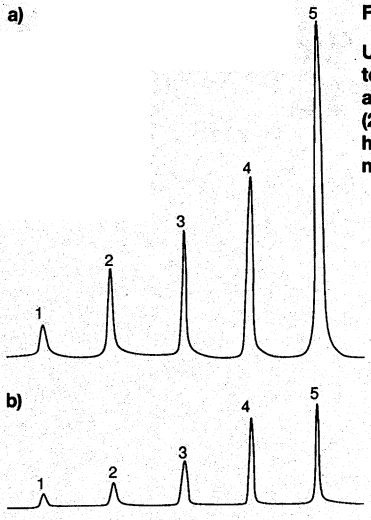

Figure 2

Undeveloped statically spotted monensin sodium spots at 150°C (1 µL each), 1–5 (21–346 ng): a) resin kettle heating chamber; b) commercial VPF Chamber.

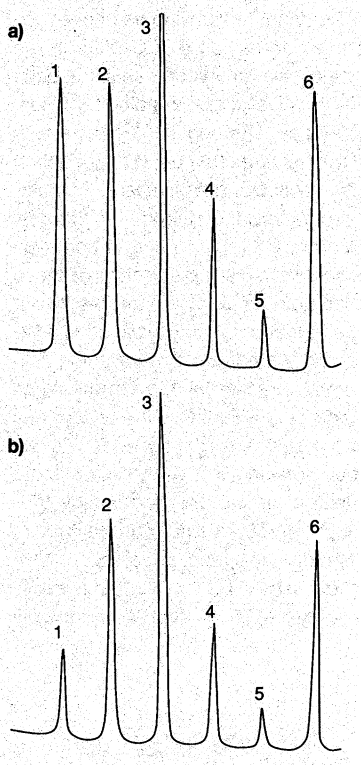

Figure 3

Undeveloped statically spotted lipids at 150°C (1 µL each of the following concentrations): 1 L-α-phosphatidylcholine (4.81 µg); 2 L-α-phosphatidylethanolamine (7.3 µg); 3 L-α-phosphatidylinositol (11.0 µg); 4 oleic acid (4.94 µg); 5 triolein (4.91 µg); 6 cholesterol (5.08 µg): a) resin kettle heating chamber; b) commercial VPF Chamber.

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References

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[2] *I. R. Kupke* and *S. Zeugner,* J. Chromatogr. **146** (1978) 261–271.

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